

DATA EVALUATION RECORD

1. Chemical: Glyphosate
2. Test Material: Technical glyphosate - 96.7%, Roundup - 41 %, /
Surfactant (MON 0818 - 100%)
3. Study Type: Acute Aquatic Studies
4. Study ID: Folmar, L.C.; Sanders, H.O.; Julin A.M. (1979)
Toxicity of the Herbicide Glyphosate and Several
of its Formulations to Fish and Aquatic Invertebrates.
USDI, FWS, Columbia National Fishery Research
Laboratory, Columbia, MO, Archives of Environmental
Contamination and Toxicology 8, 269-278 MCOGLY16.
5. Reviewed By: Dennis J. McLane Signature: *Dennis J. McLane*
Wildlife Biologist Date: 10-9-85
HED/EEB Review Time: 8 hours
CM2
557-7560
6. Approved By: Raymond W. Matheny Signature: *Raymond W. Matheny*
Section 1, Head Date: 10-10-85
HED/EEB
7. Conclusion:

Four fish studies on technical material meet the guideline requirements: rainbow trout, fathead minnow, channel catfish, and bluegill. Also, ten tests covering the four same four species and Daphnia with the product, Roundup, meet formulated product testing requirement. In addition the same four fish species were tested in the surfactant, MON 0818, These tests were also found to be sufficient. Other special tests on life stages, aged formulated product testing, various pH values, and temperatures are not required at this time.

8. Recommendations:

N/A



2009151

9. Background:

EEB discovered this study when addressing the submission for forest use. It has demonstrated the original Roundup surfactant resulted in a formulation which is much higher in toxicity than technical glyphosate.

10. Discussion of Individual Test:

N/A

11. Materials and Methods: (Excerpted from published article.)

A. Experimental Animals and Test Chemicals

Invertebrates used in acute toxicity tests were first instar daphnids (Daphnia magna), mature scuds (Gammarus pseudolimnaeus), and early fourth instar midge larvae (Chironomus plumosus); they were maintained in cultures at the Columbia National Fisheries Research Laboratory. The later instar nymphs of the mayfly (Ephemera walkeri) used in the avoidance experiments were collected from Clear Creek, near Georgetown, CO.

Test fish were rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas), channel catfish (Ictalurus punctatus), and bluegills (Lepomis macrochirus). The fish were obtained from federal fish hatcheries and were held under laboratory conditions as described by Brauhn and Schoettger (1975).

Monsanto Agricultural Products Company, St. Louis, MO, supplied the technical grade glyphosate, the isopropylamine salt of glyphosate (480.42 g/L active ingredient), the Roundup formulation with surfactant (360.32 g/L active ingredient), and the Roundup surfactant.

B. Acute Toxicity Tests

Most of the acute toxicity tests were conducted at the Columbia laboratory according to methods recommended for static toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). The fish weighed from 0.5 to 2.2 g, except those used in life-stage studies. Ten fish were exposed at each test concentration, except when the average weight of the fish exceeded 1.5 g. For the larger fish, a second series of containers was used to maintain loadings of less than 1 g of fish per liter of test solution. Technical grade glyphosate was added directly to reconstituted water containing the fish. Roundup and the Roundup surfactant were first diluted in water, then pipetted into the test containers. Acute toxicity was measured as the 48-hr EC₅₀ (effective concentration causing immobilization in 50% of test organisms) for daphnids and midge larvae, and 24- and 96-hr LC₅₀ (concentration lethal to 50% of test organisms) for the scuds and fish. The method of Litchfield and Wilcoxon (1949) was used to calculate EC₅₀'s and LC₅₀'s and their 95 percent confidence limits. All concentrations reported in the text and tables were based on active ingredients.

Early life stages (eggs, sac fry, and swim-up fry) of rainbow trout and channel catfish were tested for sensitivity to Roundup in 96-hr static toxicity tests in reconstituted water at 12 °C and 22 °C. Also, to simulate actual field exposure, eggs, sac fry, and swim-up fry were exposed to Roundup for 4 hr under static conditions, then they were transferred to fresh flowing water to observe post-treatment effects.

Reconstituted water (pH 7.2 hardness 40 mg/L as CaCO_3) was used in the static toxicity tests. Temperatures of test solutions were maintained by a controlled temperature water bath. Scuds and trout were tested at 7.12, or 17 °C, and daphnids, midge larvae and warmwater fish at 17.22 or 27 °C. The influence of pH on the toxicity of glyphosate, Roundup and the Roundup surfactant was determined in reconstituted water to which buffer salts were added that maintained the desired pH's of 6.5, 7.5, 8.5, and 9.5, Marking (1975). Test solutions were monitored and adjusted daily to the initial pH.

Changes in toxicity of Roundup aged in water were determined by simultaneous introduction of test fish into fresh solutions of Roundup and similar solutions that had been aged for 1, 3, and 7 days. Methods used to assess toxicity were the same as those described for the standard static tests.

12. Reported Results:

(Excerpted from citation abstract)

Acute toxicities for Roundup ranged from 2.3 mg/L (96-hr LC_{50} , fathead minnow) to 43 mg/L (48-hr EC_{50} , mature scuds). Toxicities of the surfactant were similar to those of the Roundup formulation. Technical glyphosate was considerably less toxic than Roundup or the surfactant; for midge larvae, the 48-hr EC_{50} was 55 mg/L and for rainbow trout, the 96-hr LC_{50} was 140 mg/L. Roundup was more toxic to rainbow trout and bluegills at the higher test temperatures, and at pH 7.5 than at pH 6.5. Toxicity did not increase at pH 8.5 or 9.5. Eyed eggs were the least sensitive life stage, but toxicity increased markedly as the fish entered the sac fry and early swim-up stages. The aging of Roundup test solutions for 7 days did not reduce toxicity to midge larvae, rainbow trout, or bluegills.

(Excerpted from study)

In static tests, the acute toxicities of Roundup varied from 2.3 mg/L for fathead minnows to 43 mg/L for mature scuds (table 1). However, the toxicities determined for other aquatic organisms were nearer to the values for fathead minnows than to those for the more resistant scuds. Toxicities of the surfactant were roughly similar to those of Roundup, whereas the contribution of technical glyphosate to the toxicity of Roundup ranged from only 29 percent for fathead minnows to 33 percent for midge larvae (table 2). These results suggest that

These results suggest that the surfactant did not merely increase the biological activity of glyphosate but was itself the primary toxic agent in Roundup. The low solubility of technical glyphosate in water (19% w/v) could account for some of the variation in LC₅₀'s obtained in acute tests.

Table 1. Toxicity of Roundup to Aquatic Invertebrates and Fish LC₅₀ or EC₅₀^a (mg/L) and 95% Confidence Limits (4174-I)

Chemical and Organism	Temp (C°)	24 hr	48 hr	96 hr
✓ Daphnids	22		3.0 (2.6-3.4)	
✓ Scuds	12	= 100	62 (40-98)	43 (28-66)
✓ Midge larvae	22		18 (9.4-32)	
Rainbow trout	12	8.3 (7.0-9.9)		8.3 (7.0-9.9)
✓ Fathead minnows	22	2.4 (2.0-2.9)		2.3 (1.9-2.8)
✓ Channel catfish	22	13 (11-16)		13 (11-16)
Bluegills	22	6.4 (4.8-8.6)		5.0 (3.8-6.6)

^a Daphnid and midge toxicities expressed as 48-hr EC₅₀ (concentration immobilizing 50% of the test organisms).

Exposure of early life-stages of rainbow trout and channel catfish to Roundup indicated that the egg stage was the least sensitive for both species (table 3). Toxicity of Roundup increased for both species at the sac fry and early swim-up stages, but decreased in the fingerling stage as the fish grew larger. To simulate actual field exposure, rainbow trout eggs and sac fry were exposed to Roundup for 6 hours. The data from these tests show a significant reduction ($P \leq 0.05$) in the hatch of trout eggs at 10 and 20 mg/L. Survival of sac fry was reduced at a concentration of 5.0 mg/L (table 4.) The absence of detectable changes in fecundity or gonadosomatic index in treated adult rainbow trout indicated that short-term exposures

Table 2. Toxicity of Roundup to Aquatic Invertebrates and Fish

		<u>LC₅₀ or EC₅₀^a (mg/L) and 95% Confidence Limits</u>		
<u>Chemical and Organism</u>	<u>Temp (C°)</u>	<u>24 hr</u>	<u>48 hr</u>	<u>96 hr</u>
<u>Glyphosate</u>				
✓ Midge larvae	22		55 (31-97)	
✓ Rainbow trout	12	140 (120-170)		140 (120-170)
✓ Fathead minnows	22	97 (79-120)		97 (79-120)
✓ Channel catfish	22	130 (110-160)		130 (110-160)
Bluegills	22	150 (120-190)		140 (110-160)
<u>Surfactant</u>				
Midge larvae	22		13 (7.1-24) ✓	
Rainbow trout	12	2.1 (1.6-2.7)		2.0 (1.5-2.7)
Fathead minnows	22	1.4 (1.2-1.7)		1.0 (1.2-1.7)
Channel catfish	22	18 (8.5-38)		13 (10-17)
Bluegills	22	3.0 (2.5-3.7)		3.0 (2.5-3.7)

^a Midge toxicity expressed as EC₅₀ (concentration immobilizing 50% of the test organisms).

should not be detrimental to gonadal maturation; however, due to the increased susceptibility of the early life stages, application of Roundup should be avoided or caution should be exercised when it is applied during seasons when young fish may be present in receiving waters.

Influence of Temperature, pH, and Aged Water Solutions on Toxicity

In static tests, the toxicity of Roundup to rainbow trout and bluegills increased with increasing temperature (table 5). Roundup was about twice as toxic to rainbow trout at 17 °C than at 7 °C. It was also more toxic to bluegills at 27 °C than

Table 3. Toxicity of Roundup to Various Life-Stages of Rainbow Trout and Channel Catfish

Organism and Life-Stages	<u>LC₅₀ (mg/L and 95% Confidence Limits</u>	
	24 hr	96 hr
<u>Rainbow trout</u>		
Eyed Eggs	46 (35-61)	16 (13-19)
Sac fry	11 (8.8-13)	3.4 (2.2-5.3)
Swim-up fry	2.4 (2.0-2.9)	2.4 (2.0-2.9)
Fingerling	2.2 0.93-5.2)	1.3 (1.1-1.6)
✓Fingerling (1 g)	8.3 (7.0-9.9)	8.3 (7.0-9.9)
<u>Channel Catfish</u>		
Eyed eggs	43 (36-51)	
Sac fry	4.3 (3.6-5.1)	4.3 (3.6-5.1)
Swim-up fry	3.7 (3.4-4.1)	3.3 (2.8-3.9)
Fingerling (2.2 g)	13 (11-16)	13 (11-16)

a Not determined.

at 17 °C. The increased toxicity of Roundup to fish in warm water should not pose a hazard to fish in western irrigation canals because the herbicide is ordinarily applied in the late fall when the water is relatively cool. However, this may not be true for warmer littoral areas in impounded waters where higher temperatures may impose toxicity problems along treated shorelines.

Table 4. Survival of Eyed Eggs and Sac Fry of Rainbow Trout in Fresh Flowing Water after a 4-Hr Exposure to Roundup

Toxicant concentration (mg/L)	Eyed eggs hatched (%)	Survival of sac fry to swim-up fry (%)
Control	84	98
2.0	74	89
5.0	75	54a
10.0	69a	0a
20.0	70a	0a

a Significant differences by Student's t-test ($P < 0.05$).

Table 5. Effects of Temperature on the Toxicity of Roundup to Two Species of Fish

Organism and Temp (°C)	LC ₅₀ (mg/L) and 99% Confidence Limits	
	24 hr	96 hr
<u>Rainbow trout</u>		
7°	14 (11-17)	14 (11-16)
12°	14 (11-17)	7.5 (6.3-9.0)
17°	7.5 (6.3-9.0)	7.4 (6.2-8.9)
<u>Bluegills</u>		
17°	9.6 (7.9-12.0)	7.5 (6.3-9.0)
22°	6.4 (4.8-8.6)	5.0 (3.8-6.6)
27°	4.3 (3.4-5.4)	4.0 (3.2-5.0)

Roundup was more toxic to rainbow trout and bluegill at pH 7.5 than at pH 6.5 (table 6); however, the toxicity did not increase significantly at pH 8.5 and 9.5. Technical glyphosate was also less toxic to fish at a higher pH, but the surfactant appeared to be more toxic at a higher pH. In western irrigation systems where pH ranges between 7.5 to 8.0, the expected 96-hour LC_{50} for rainbow trout would be about 1.5 mg/L. Although this chemical is more toxic at higher pH's a hundred-fold safety factor over expected water concentrations of glyphosate still remains.

Solutions of Roundup aged for up to 7 days in reconstituted water at 12 °C and 22 °C did not change in toxicity to midge larvae, rainbow trout, or bluegills, (table 7). In irrigation canals resident fish and aquatic invertebrates would be exposed for only short periods of time; however, under static conditions such as those encountered in fish rearing ponds, reapplication of the chemical within short time intervals may cause accumulation of the chemical to toxic levels.

Table 6. Effects of pH on Toxicity of Roundup, Glyphosate, and the Surfactant to Rainbow Trout and Bluegills

Chemicals Organism, and pH	LC ₅₀ (mg/L) and 95% Confidence Limits	
	24 hr	96 hr
<u>Roundup</u>		
Rainbow trout		
6.5	14 (12-17)	7.6 (6.4-9.1)
7.5	2.4 (2.0-2.9)	1.6 (1.2-2.2)
8.5	2.4 (2.0-2.9)	1.4 (1.2-1.7)
9.5	2.4 (2.0-2.9)	1.4 (1.2-1.7)
Bluegills		
6.5	7.6 (6.4-9.1)	4.2 (3.5-5.0)
7.5	4.0 (3.2-5.0)	2.4 (2.0-2.9) ✓
8.5	3.9 (3.1-4.9)	2.4 (2.0-2.9) ✓
9.5	2.4 (2.0-2.9)	1.8 (1.3-2.5)
<u>Glyphosate</u>		
Rainbow Trout		
6.5	240 (200-290)	140 (120-170)
9.5	240 (200-290)	240 (200-290)
Bluegills		
6.5	240 (200-290)	140 (120-170)
9.5	230 (190-280)	240 (170-280)
<u>Surfactant</u>		
Rainbow trout		
6.5	7.4 (6.2-8.9)	7.4 (6.1-9.0)
9.5	1.4 (1.2-1.7)	0.65 (0.54-0.78)
Bluegills		
6.5	4.2 (3.1-5.7)	1.3 (1.1-1.6)
9.5	3.0 (2.2-4.1)	1.0 (0.72-1.4)

Table 7. Toxicity of Fresh and Aged Roundup Solutions to Midge Larvae, Rainbow Trout, and Bluegills in Static Tests

Organism and Days Aged	LC ₅₀ and EC ₅₀ (mg/L) and 95% Confidence Limits		
	24 hr	48 hr	96 hr
Midge larvae ^a			
0	<100	43 (18-53)	
1	<100	34 (25-45)	
3	<100	34 (10-65)	
7	<100	30 (12-77)	
Rainbow trout			
0	19 (16-32)		9.0 (7.5-11)
1	14 (11-16)		7.6 (6.4-9.1)
3	14 (11-16)		7.6 (6.4-9.1)
7			
Bluegills			
0	4.3 (3.4-5.5)		4. (2.9-5.5)
1	6.6 (4.8-9.0)		6.0 (5.6-6.5)
3	8.0 (6.4-10.0)		7.0 (5.0-9.8)
7	6.2 (4.6-8.4)		5.6 (4.0-7.8)

^a Toxicities expressed as 48 hr EC₅₀.

13. Study Author's Conclusion/QA Measures

Study author's conclusions are reported under No. 7.
Conclusions.

The report did not specifically address the QA measures other than those procedures reported under No. 11. Materials and Methods.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures:

The following items were not reported or did not meet guideline requirements.

1. Fourth rather than first instar midges were used in the technical glyphosate test.

2. The acclimation period was not reported for any of the tested species.

3. Only four species were tested in technical glyphosate (see table 2).

4. Raw data are available only for 1 g rainbow trout tested in Roundup - 41 percent glyphosate.

5. Mature rather than first instar scuds were tested.

6. Life stages testing is not required.

7. Testing aged formulated product is not required.

8. 96-hour testing at different pH values and temperature

B. Statistical Analysis:

The article stated Litchfield and Wilcoxon method of calculating the LC₅₀ was used. This is an acceptable method. The one set of raw data (1 g rainbow trout-Roundup) was verified by the EEB computerized binominal method and found the reported LC₅₀ sufficient (see attached).

C. Discussion/Results:

Only those species listed in table 2 under glyphosate were tested with technical glyphosate. The midge larvae are fourth instar rather than first instar. Therefore, only the rainbow trout, fathead minnows, channel catfish, and bluegill meet the minimum testing requirements of the guidelines.

Therefore, of the remaining acute studies with the fourth instar midges, mature scuds, eyed eggs, sac fry, and swim-up fry for rainbow trout and catfish; 1- and 3-day age formulated product testing; and pH change testing will not be required.

The following tests meet the minimum requirements for acute aquatic testing on the technical grade material: (96.7%)

<u>Organism</u>	<u>LC₅₀ (95% CL) (mg/L)</u>
Rainbow trout	140 (120-170)
Fathead minnow	97 (79-170)
Channel catfish	130 (110-160)
Bluegill	140 (110-160)

CORE ✓
✓

In addition, the following tests may be used to meet a portion of the requirements formulated product testing:

<u>Chemical and Organisms</u>	<u>LC₅₀ (95% CL) mg/L</u>	
	<u>48 hours</u>	<u>96 hours</u>
Roundup (41%)		
<u>Daphnia magna</u>	3 (2.6-3.4)	
Fathead minnows ✓		2.3 (1.9-2.8)
Channel catfish ✓		13 (11-16)
Bluegills		5.0 (3.8-6.6)
Rainbow trout		8.3 (7.0-9.9)
Rainbow trout		7.5 (6.3-9.0)
Rainbow trout		1.6 (1.2-2.2)
Rainbow trout (.5 g) ✓		1.3 (1.1-1.6)
Rainbow trout		9 (7.5-11)
Bluegill		4 (2.9-5.5)
Bluegill ✓		2.4 (2.0-2.9)

CORE

These studies on the surfactant are adequate.

Chemical and Organisms	<u>LC₅₀ (95% CL) mg/L</u>	
	48 hours	96 hours
MON0818 Surfactant		
Rainbow trout		2.0 (1.5-2.7)
Rainbow trout		7.4 (6.1-9.0)
Rainbow trout		.65 (0.54-0.78)
Fathead minnow		1.0 (1.2-1.7)
Channel catfish		13 (10-17)
Bluegill		3.0 (2.5-3.7)
Bluegill		1.3 (1.1-1.6)
Bluegill		1 (.72-1.4)
Bluegill		3.0 (2.5-3.7)

D. Adequacy of Study:

1. Classification: Four tests on the technical material meet the minimum guideline requirements for testing on fish both cold water(1)and warm water(3)(core). Eleven tests meet the requirement for formulated product when a highly toxic surfactant has been used (core) for invertebrates(1), cold water(5), and warm water(6)species. Eight fish studies for both cold water (3) and warm water(5) species testing of a highly toxic surfactant are sufficient for hazard assessment (supplemental). (See pages 13 - 14 for listings of specific test organisms and LC50 value.)

2. Rationale: The testing in the following situations does not meet any present requirement:
 - a. Fish life-stage, such as eyed eggs, sac fry, and swim-up fry
 - b. Aged formulated product testing
 - c. Testing different pH and temperature
 Tests on these life stages do not meet guideline requirements.
 - a. Fourth instar midges
 - b. Mature scuds

These studies are sufficient for hazard assessment but no requirement has been established (supplemental).

3. Repairability: N/A

15. Completion of One-Liner for Study:

One-liner form completed.

16. CBI Appendix:

N/A

MCLANE ROUNDUP *Rainbow Trout 96LC50*
~~MEQDE 48-11-50~~

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
11.2	10	10	100	.0976563
6.4	10	0	0	.0976563

THE BINOMIAL TEST SHOWS THAT 6.4 AND 11.2 CAN BE
 USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
 CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
 ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.46641

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
 PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
 NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

RECORD OF TELEPHONE CALL OR VISITOR

INCOMING CALL ☒

OUTGOING CALL ☐

VISITOR ☐

DATE

TIME

1-6-3 2:10

NAME OF PERSON

J. Wahn

NAME & ADDRESS OF COMPANY

Columbia Lab

COMPANY TEL. NO. (Include Area Code)

276-5399

REGISTRATION NO. OR FILE SYMBOL

DATE OF LATEST SUBMISSION

BRIEF SUMMARY OF CONVERSATION

I requested the purity of MON 818 when tested by Tolmar et al. (1979). It is 100% technical as reported in the Columbia Hand book

ACTION TAKEN

RECORDED BY (Name)

Dennis M. Lane

REFERRED TO (Name)